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Meat Consumption During Pregnancy and Substance Misuse Among Adolescent Offspring: Stratification of *TCN2* Genetic Variants

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Background: Reducing meat consumption is often advised; however, inadvertent nutritional deficiencies during pregnancy may result in residual neurodevelopmental harms to offspring. This study assessed possible effects of maternal diets in pregnancy on adverse substance use among adolescent offspring.

Methods: Pregnant women and their 13-year-old offspring taking part in a prospective birth cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC), provided Food Frequency Questionnaire data from which dietary patterns were derived using principal components analysis. Multivariable logistic regression models including potential confounders evaluated adverse alcohol, cannabis, and tobacco use of the children at 15 years of age.

Results: Lower maternal meat consumption was associated with greater problematic substance use among 15-year-old offspring in dose–response patterns. Comparing never to daily meat consumption after adjustment, risks were greater for all categories of problem substance use: alcohol, odds ratio OR = 1.75, 95% CI = (1.23, 2.56), $p < 0.001$; tobacco use OR = 1.85, 95% CI = (1.28, 2.63), $p < 0.001$; and cannabis OR = 2.70, 95% CI = (1.89, 4.00), $p < 0.001$. Given the likelihood of residual confounding, potential causality was evaluated using stratification for maternal allelic variants that impact biological activity of cobalamin (vitamin B12) and iron. Lower meat consumption disproportionally increased the risks of offspring substance misuse among mothers with optimally functional (homozygous) variants (rs1801198) of the gene transcobalamin 2 gene (*TCN2*) which encodes the vitamin B12 transport protein transcobalamin 2 implicating a causal role for cobalamin deficits. Functional maternal variants in iron metabolism were unrelated to the adverse substance use. Risks potentially attributable to cobalamin deficits during pregnancy include adverse adolescent alcohol, cannabis, and tobacco use (14, 37, and 23, respectively).

Conclusions: Lower prenatal meat consumption was associated with increased risks of adolescent substance misuse. Interactions between *TCN2* variant status and meat intake implicate cobalamin deficiencies.

Key Words: Meat, Cobalamin, Single Nucleotide Polymorphism (SNP), Avon Longitudinal Study of Parents and Children (ALSPAC), Transcobalamin Gene (*TCN2*).

GROWING EVIDENCE INDICATES that deficiencies in critical nutrients in utero can increase risks for adverse cognitive development and behavioral problems among otherwise healthy children (Anjos et al., 2013). Consequently, it is widely accepted that nutritional deficits and toxic exposures occurring prenatally can alter early life programming and impact life time risks of psychiatric disorders through persistent epigenetic changes. Examples of such maternal deficiencies of essential nutrients include cobalamin (vitamin B12), iron, iodine, folate, and thiamine which have all been linked to enduring neurodevelopmental deficits (Kofink et al., 2013). Epigenetic changes in the environmental programming of neurodevelopmental processes are especially relevant to nutritional deficiencies in vitamin B12 because many epigenetic modifications are dependent upon alterations in DNA methylation (Caramaschi et al., 2017). Vitamin B12 is critical to the process of DNA methylation and deficiencies in vitamin B12 can cause abnormalities in DNA methylation (Caramaschi et al., 2017). The identification of such nutrients has led to successful public health prevention programs and policies, for example, the fortification of foods with folate to prevent neural tube abnormalities in many countries (Anjos et al., 2013). However, the majority

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of published studies on neurodevelopmental outcomes in childhood have focused on neither adolescent cohorts nor substance abuse-related end points.

Vegetarian dietary patterns are associated with improved health outcomes among adults and, in addition, have strong ethical imperatives including promotion of sustainability, food security, and reducing industrialized production of animals. However, avoidance of relatively nutrient-dense meats can decrease intakes of cobalamin, iron, omega-3 fatty acids, selenium, and zinc, particularly in young women of childbearing age (Fayet et al., 2014). Cobalamin is a biochemically essential nutrient available predominantly from meats and shellfish; up to 62% of vegetarians are deficient in this nutrient during pregnancy (Pawlak et al., 2013). Profound neurodevelopmental abnormalities due to severe cobalamin deficiencies were first identified among infants from Indian vegan and vegetarian mothers (Jadhav et al., 1962). Among Western populations, infants of cobalamin deficient mothers have poor brain growth, developmental regression, irritability, thrive poorly (Graham et al., 1992), and demonstrate residual deficits in cognitive and social development (Bhate et al., 2012; del Rio Garcia et al., 2009). Thus, it is of great interest to determine whether the lower cobalamin status of ovo-lacto vegetarian mothers and nonvegetarians eating less meat can result in residual impairments in the neurodevelopment of their offspring (Bonilla et al., 2012; Koebnick et al., 2004). We were particularly interested in substance use outcomes, as there are few extant reports on the issue.

Here, we hypothesized that maternal prenatal nutritional deficiencies were risk factors for adverse substance abuse among offspring. Our approach was to first evaluate whether any dietary patterns in pregnancy were associated with adverse substance abuse among offspring. We then evaluated which specific foods comprising those patterns were associated with the increased risks. Within the vegetarian dietary pattern group, lower maternal meat consumption was identified as a risk factor. We evaluated 2 candidate nutrients (cobalamin and iron) that are both frequently diminished among vegetarians and for which deficiencies have adverse neurological consequences. Finally, we stratified the maternal cohort by genetic variants that affect the status and function of these nutrients, for example, the variants affecting the bioavailability and transport of cobalamin. Within each strata of genetic variant, we examined the relationships between lower meat consumption among mothers and risks of substance use among their adolescent children.

MATERIALS AND METHODS

Participants

The sample comprised participants from the Avon Longitudinal Study of Parents and Children (ALSPAC) (Boyd et al., 2013). ALSPAC is an ongoing population-based prospective cohort study in the southwest of England. Pregnant women resident in the former Avon Health Authority (which included the city of Bristol), who had an estimated date of delivery between April 1, 1991, and December 31, 1992, were invited to take part, resulting in a cohort of 14,541 pregnancies which resulted in 13,976 singletons and twins alive at 1 year of age. Detailed information about ALSPAC is available online (http://www.bris.ac.uk/alspac) with details of all the data available (http://www.bristol.ac.uk/alspac/researchers/data-access/data-dictionary/). Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and local Research Ethics Committees.

Substance Use Outcomes at 15 Years of Age

At the age of approximately 15 years, 9,979 young people were invited to a clinic and 5,246 (52.6%) attended. Median age at attendance was 15 years 5 months, interquartile range (IQR) = 15 years 3 months to 15 years 7 months. Of the clinic attendees, 5,228 started a session where data were collected via an electronic questionnaire that captured data on current substance use behavior. Responses to questions on alcohol, cannabis, and tobacco use and associated problems were used to derive 10 substance use variables with binary classification. Five alcohol-related measures were derived, as described by Melotti and colleagues (2013), to indicate heavy typical drinking, frequent drinking, regular binge drinking, alcohol psychosocial problems, and alcohol behavioral problems. Three measures were created from questions

Table 1. Prevalence Rates for Problematic Substance Use at 15 Years of Age in Whole Population and Stratified by Gender

	Population *N* (%)	Males *N* (%)	Females *N* (%)	*p*
Alcohol measures				
Heavy typical drinking	1,024 (21.1)	441 (19.5)	583 (22.4)	0.011
Frequent drinking	974 (19.3)	487 (20.6)	487 (18.1)	0.023
Regular bingeing	523 (10.4)	252 (10.7)	271 (10.1)	0.497
Psychosocial problems	1,283 (25.7)	550 (23.6)	733 (27.5)	0.002
Behavioral problems	480 (9.5)	234 (10.0)	246 (9.1)	0.314
Cannabis measures				
Past-year use	984 (19.5)	472 (20.1)	512 (19.0)	0.335
Moderate use	485 (9.6)	247 (10.5)	238 (8.8)	0.050
Problematic use	194 (3.8)	119 (5.1)	75 (2.8)	<0.001
Tobacco measures				
Recent use	893 (17.5)	329 (13.8)	564 (20.7)	<0.001
Weekly use	528 (10.3)	195 (8.2)	333 (12.3)	<0.001

Table shows *p*-values from chi-square tests of association between gender and each binary indicator of problematic substance use.

Alcohol: *heavy typical drinking* (more than 4 drinks per occasion in the previous 6 months), *frequent drinking* (20 or more occasions in past 6 months), *regular binge drinking* (consuming 5 or more drinks in any 24-hour period in the previous 2 years on 20 or more occasions), *alcohol psychosocial problems* (any of the following events experienced on more than 3 occasions in the previous 2 years: "set a limit, drank more"; "felt should stop/cut back on drinking"; "spent a great deal of day drinking"; "not done things because of drinking"; "continued to drink despite causing problems"; "unable to keep up with other activities"; "parents/friends complained"; and "had a 'blackout' because of drinking"), and finally *alcohol behavioral problems (similar to psychosocial but based on following 4 events:* "used alcohol in dangerous situations"; "been accidentally physically hurt while drinking"; "had a problem with the police"; and "got into fights because of drinking").

Cannabis: *any past-year cannabis use* (any reported use in the last 12 months), *moderate use* (used cannabis at least occasionally), and finally *problematic cannabis use* (a report of fairly often/very often to 1 or more of: smoking before midday, smoking when alone, having memory problems, reproaches from family, unsuccessful attempts to quit, or problems linked to cannabis consumption).

Tobacco: Two measures were created to indicate: *recent tobacco use* (any smoking of cigarettes in the last 30 days) and *weekly tobacco use* (currently smoking on a weekly basis).

which included the Cannabis Abuse Screening Test (Piontek et al., 2008) to indicate any past-year cannabis use, moderate use, and problematic cannabis use. Finally, 2 measures were created to indicate recent tobacco use and current weekly tobacco use (Table 1).

Maternal Diet in Pregnancy

Foods consumed were assessed by a self-administered Food Frequency Questionnaire (FFQ) sent to the mothers during the third trimester of their pregnancy. A wide range of foods were listed with response options "never/rarely," "once in 2 weeks," "1 to 3 times/wk," "4 to 7 times/wk," and "more than once a day." Median gestation at completion was 32 weeks, IQR = 32 weeks to 33 weeks of gestation. Comparable measures of intake within each food category were derived in relation to the child's own diet from FFQ responses collected at 13 years of age.

Dietary Patterns. As previously reported (Northstone et al., 2008), the dietary information in pregnancy from the FFQ was used to derive scored for 5 continuous orthogonal dietary pattern categories using principal component analysis ("health conscious," "traditional," "processed," "confectionary," and "vegetarian").

Food Categories. Ordinal measures of food consumption for food categories were derived from responses to 1 or more questions. Increasing frequency of meat consumption was derived from 5 questions covering: sausages/burger, meat pies, red meat, poultry, and offal. Individual frequency data were coded as follows: "never/rarely" = 0, "once in 2 weeks" = 0.5, "1 to 3 times per week" = 2.0, "4 to 7 times per week" = 5.5, and "more than once a day" = 10, before summing across responses and collapsing into 5 large categories based on the resulting distribution. These categories are roughly interpreted as quantity of meat/fish consumption per fortnight (2 weeks) (Fig. 1). Further measures were created from single question responses including consumption of fresh fruit, soy ("Soya 'Meat,' T V P, Vegeburgers"), and pulses ("Dried peas, beans, lentils, chick peas"). For each of these variables, we collapsed over rare response categories.

Vegetarianism. Responses to "are you or have you ever been a vegetarian?" included "yes, I am now" (coded as 1), "yes, in past not now [i.e., during the pregnancy]," and "no, never" (both coded as 0).

Other Covariates

Sociodemographic Variables. Early life social and economic factors that were related to both substance use patterns at 15 years of age (Melotti et al., 2013) and maternal dietary patterns (Northstone et al., 2008) were identified. Data collected by questionnaire during the antenatal period included variables for housing tenure, highest maternal education level achieved, parity, parental social class, occupations, ethnicity of young person, and home overcrowding at enrollment. Factors after the birth included: maternal age at delivery and when the child was a toddler; and quintiles of household disposable income taking account of family size and composition and estimated housing benefits.

Parent–child relationship measures. The computerized session within the 15-year clinic also contained questions from the Edinburgh Scale of Youth Transitions and Crime (Smith et al., 2001). Measures pertaining to the relationship between the young person and their parents which have been previously shown to be strong predictors of substance use in this age group were constructed. More details can be found in the footnote to Table S5.

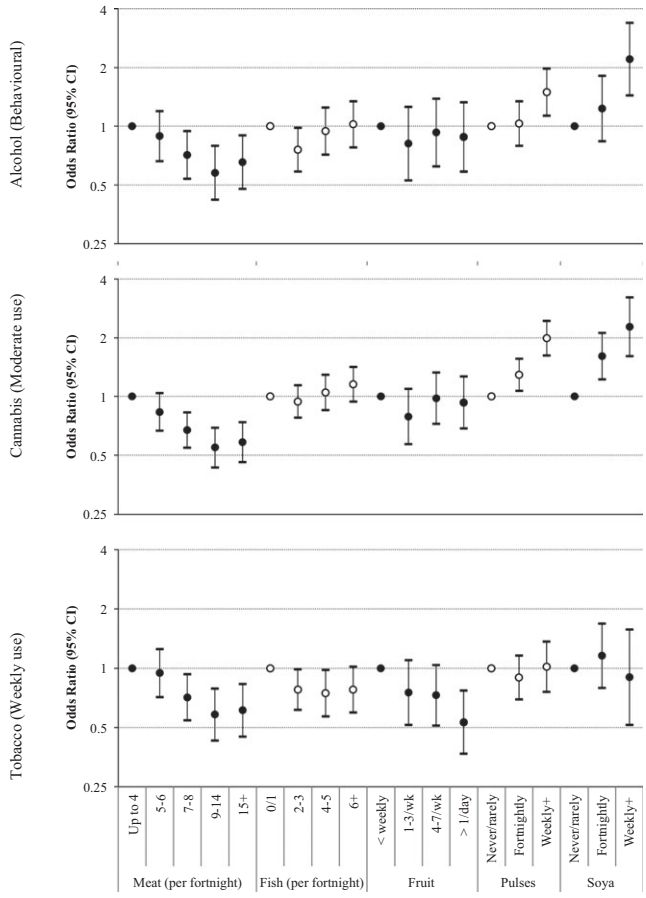

Fig. 1. Unadjusted associations between maternal food categories and odds of substance use outcomes among their adolescent children. Circles and bars represent odds ratios and 95% confidence interval (95% CI) of unadjusted relationships between maternal consumption of selected foods and risk of substance use among their adolescent children.

Statistical Methods

First, a series of univariable logistic regression models were estimated for combination of binary substance use outcomes and continuous standardized dietary pattern score. These models were adjusted for the potential confounding effect of the sociodemographic factors described above. We then selected those dietary patterns for which strong associations remained postadjustment (namely "healthy" and "vegetarian")—from these, we determined which individual food items were strongly associated (factor loading >0.5) with these patterns. These data (originally published in Northstone et al., 2008) are shown in Table S1. Fish, non–white bread, pasta, rice, fresh fruit, meat, meat substitutes, pulses, and nuts were selected for further study. Analyses were subsequently repeated for these same food categories using food frequency data on the child's own diet at 13 years of age. Missing data considerations are detailed in Supplementary materials (Appendix S1). All regression modeling was carried out in Stata version 13.1 (StataCorp LP, College Station, TX).

Attributable Risk

Transcobalamin 2 (*TCN2*) genotyping was conducted as previously described (Bonilla et al., 2012). For each outcome, we calculated the attributable risk, both in the whole population and stratified by *TCN2* genotype. Exposed individuals are those with less than regular meat consumption, in other words, being in

categories 1 to 4 of the 5-category meat consumption measure. This cutoff was chosen as the most conservative estimation of attributable risk of lower meat consumption. Attributable risk was calculated as the difference in risk of each outcome in those exposed and not exposed, expressed as a proportion of all exposed cases.

RESULTS

Gender Differences and Social Patterning in Rates of Substance Use at 15 Years of Age

Of the 5,228 young people who commenced the clinic computing session, 5,109 (97.7%) provided sufficient information on substance use outcomes. There was moderate evidence for gender differences in substance use measures (Table 1). The 3 outcomes of alcohol behavioral problems, moderate cannabis use, and weekly tobacco use were present in approximately 10% of the cohort; they represented significant adverse use of each substance and became the focus of this report. Recent tobacco use is strongly and consistently socially patterned, while for the other 2 substances, associations are weaker and less consistent (Table 2). Table S2 shows the association between social and economic factors and each dietary pattern measure. Analyses regarding the remaining 7 substance use outcomes, dietary patterns, and individual foods are presented in Table S3.

Maternal Diet and Adolescent Substance Use

Table 3 shows the results from regression analyses for the binary outcomes behavioral alcohol problems, moderate cannabis use, and weekly tobacco use (see Table S3 for additional outcomes). Results show little evidence of an association between the "traditional," "processed," or "confectionary" patterns and any of the 3 substance use outcomes. For the pattern labeled "health conscious," there is weak evidence of an *increase* in the odds of alcohol behavior problems following adjustment for confounding, strong evidence for a more substantial increase in odds of occasional cannabis use, and evidence of *reduced* odds of weekly tobacco use which was heavily confounded. In contrast, the dietary pattern labeled "vegetarian" showed consistent and strong evidence of a moderate increase in the odds of all 3 substance use outcomes and associations. These associations were strengthened after adjustment. The "vegetarian" dietary pattern was also associated with "heavy typical drinking" and "alcohol psychosocial problems" in unadjusted analyses (Table S3). In moderate agreement with the dietary pattern results, self-reported vegetarianism was seen to be associated with increased odds of alcohol problems and cannabis use, but was not associated with weekly smoking.

Table 2. Associations Between Socioeconomic Characteristics and the 3 Key Adverse Substance Use Outcomes

Risk factor	Category	Alcohol behavioral problems (%)	Cannabis moderate use (%)	Tobacco weekly use (%)
Ethnicity	White	454 (9.5)	452 (9.4)	497 (10.2)
	Nonwhite	23 (10.2)	29 (12.8)	26 (11.4)
	p	0.705	0.090	0.570
Maternal age at delivery	<25 years	88 (11.6)	74 (9.6)	120 (15.5)
	25 to 29	173 (8.9)	157 (8.1)	206 (10.5)
	30 to 34	157 (9.2)	163 (9.5)	148 (8.6)
	35+ years	62 (9.7)	91 (14.2)	54 (8.4)
	p	0.192	<0.001	<0.001
Housing tenure	Mortgaged/owned	364 (8.8)	373 (9)	386 (9.3)
	Privately rented	38 (10.7)	41 (11.6)	44 (12.2)
	Subsidized housing	62 (15.4)	53 (13.1)	74 (18.0)
	p	<0.001	0.012	<0.001
Parity	1st child	193 (8.1)	201 (8.4)	219 (9.0)
	2nd	186 (11.0)	174 (10.3)	181 (10.6)
	3rd or greater	82 (10.4)	94 (11.8)	97 (12.0)
	p	0.004	0.008	0.031
Maternal education[a]	>O-level	183 (8.1)	233 (10.3)	164 (7.2)
	O-level	179 (10.6)	145 (8.5)	212 (12.4)
	<O-level	103 (11.0)	94 (10.0)	127 (13.4)
	p	0.007	0.147	<0.001
Equivalized income	Top 20%	98 (9.1)	101 (9.3)	79 (7.2)
	Middle 60%	243 (9.0)	253 (9.4)	278 (10.2)
	Bottom 20%	80 (12.4)	77 (11.9)	93 (14.1)
	p	0.027	0.131	<0.001
Parental social class	Professional	52 (6.5)	70 (8.8)	43 (5.4)
	Managerial/technical	197 (9.3)	216 (10.1)	212 (9.9)
	Skilled nonmanual	100 (9.2)	87 (7.9)	120 (10.8)
	Skilled manual or lower	78 (13.1)	61 (10.2)	93 (15.3)
	p	0.001	0.172	<0.001
Home over crowding	≤1 person/room	437 (9.4)	432 (9.3)	454 (9.6)
	>1 person/room	26 (14.6)	28 (15.7)	44 (24.4)
	p	0.020	0.004	<0.001

Table shows chi-square tests of association between demographic measures and each binary indicator of problematic substance use.

[a]O-levels were an examination taken around the age of 16 years at the end of compulsory schooling. They have been replaced by general certificates of secondary education.

Table 3. Associations Between Maternal Dietary Measures in Pregnancy and Adverse Substance Use Outcomes in Offspring at 15 Years of Age

	Alcohol (behavioral)		Cannabis (moderate)		Tobacco (weekly use)	
	Unadjusted	Adjusted for SES	Unadjusted	Adjusted for SES	Unadjusted	Adjusted for SES
Dietary patterns						
"Health Conscious"	0.96 [0.87, 1.07]	1.13 [0.99, 1.28]	1.21 [1.09, 1.33]	1.29 [1.14, 1.47]	0.75 [0.68, 0.83]	0.91 [0.80, 1.04]
p	0.468	0.067	<0.001	<0.001	<0.001	0.175
"Traditional"	0.98 [0.89, 1.09]	0.96 [0.86, 1.08]	0.98 [0.89, 1.09]	0.97 [0.86, 1.08]	1.04 [0.94, 1.14]	1.01 [0.90, 1.12]
p	0.752	0.486	0.711	0.574	0.436	0.978
"Processed"	1.02 [0.92, 1.13]	0.94 [0.83, 1.07]	1.02 [0.92, 1.13]	1.00 [0.88, 1.14]	1.11 [1.01, 1.22]	0.96 [0.84, 1.08]
p	0.704	0.362	0.750	0.997	0.036	0.482
"Confectionary"	1.02 [0.92, 1.13]	1.07 [0.95, 1.19]	0.91 [0.82, 1.01]	0.94 [0.84, 1.06]	0.98 [0.88, 1.08]	0.97 [0.87, 1.08]
p	0.689	0.259	0.072	0.306	0.618	0.584
"Vegetarian"	1.22 [1.12, 1.32]	1.28 [1.17, 1.41]	1.37 [1.27, 1.48]	1.42 [1.30, 1.55]	1.15 [1.06, 1.25]	1.21 [1.10, 1.33]
p	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Food categories						
Meat (ref: 0 to 4/fortnight)						
5 to 6 per fortnight	0.89 [0.66, 1.20]	0.92 [0.66, 1.28]	0.70 [0.53, 0.93]	0.73 [0.53, 1.00]	0.95 [0.71, 1.25]	0.95 [0.68, 1.31]
7 to 8 per fortnight	0.71 [0.54, 0.94]	0.67 [0.49, 0.92]	0.52 [0.40, 0.68]	0.50 [0.37, 0.67]	0.71 [0.54, 0.93]	0.63 [0.46, 0.87]
9 to 14 per fortnight	0.58 [0.42, 0.79]	0.53 [0.37, 0.76]	0.42 [0.31, 0.57]	0.41 [0.29, 0.58]	0.58 [0.43, 0.79]	0.54 [0.38, 0.76]
15+ per fortnight	0.66 [0.48, 0.90]	0.57 [0.39, 0.81]	0.44 [0.32, 0.60]	0.37 [0.25, 0.53]	0.61 [0.45, 0.83]	0.54 [0.38, 0.78]
p	0.003	0.001	<0.001	<0.001	<0.001	<0.001
Fish (ref: 0/1 per fortnight)						
2 to 3 per fortnight	0.76 [0.59, 0.98]	0.77 [0.57, 1.03]	0.84 [0.65, 1.09]	0.81 [0.60, 1.09]	0.78 [0.62, 0.99]	0.88 [0.67, 1.17]
4 to 5 per fortnight	0.95 [0.72, 1.25]	1.17 [0.85, 1.60]	1.04 [0.79, 1.37]	1.10 [0.80, 1.50]	0.75 [0.57, 0.98]	1.02 [0.75, 1.39]
6+ per fortnight	1.02 [0.78, 1.34]	1.20 [0.88, 1.64]	1.17 [0.89, 1.53]	1.11 [0.81, 1.51]	0.78 [0.60, 1.02]	1.04 [0.76, 1.42]
p	0.110	0.017	0.118	0.140	0.086	0.685
Fresh fruit (ref: <weekly)						
1 to 3 times a week	0.82 [0.53, 1.26]	0.83 [0.50, 1.36]	0.79 [0.51, 1.24]	0.96 [0.55, 1.67]	0.75 [0.52, 1.10]	1.03 [0.64, 1.66]
4 to 7 times a week	0.93 [0.62, 1.39]	1.10 [0.68, 1.76]	1.02 [0.67, 1.53]	1.30 [0.77, 2.19]	0.73 [0.51, 1.04]	1.14 [0.72, 1.79]
More than once per day	0.88 [0.59, 1.33]	1.13 [0.69, 1.84]	1.03 [0.68, 1.56]	1.26 [0.74, 2.16]	0.53 [0.37, 0.77]	0.88 [0.55, 1.42]
p	0.732	0.266	0.279	0.244	0.002	0.295
Pulses (ref: never/rarely)						
Fortnightly	1.03 [0.79, 1.34]	1.23 [0.91, 1.68]	1.58 [1.24, 2.02]	1.85 [1.39, 2.45]	0.90 [0.69, 1.16]	1.24 [0.92, 1.69]
Weekly+	1.50 [1.14, 1.98]	1.92 [1.38, 2.68]	2.41 [1.87, 3.11]	2.96 [2.19, 4.01]	1.02 [0.76, 1.37]	1.53 [1.08, 2.17]
p	0.016	0.001	<0.001	<0.001	0.691	0.040
Soy (ref : never/rarely)						
Fortnightly	1.23 [0.84, 1.82]	1.25 [0.81, 1.93]	1.54 [1.07, 2.20]	1.43 [0.95, 2.14]	1.16 [0.80, 1.69]	1.45 [0.96, 2.19]
Weekly+	2.21 [1.44, 3.39]	2.48 [1.55, 3.98]	2.98 [2.00, 4.43]	3.11 [2.03, 4.77]	0.90 [0.52, 1.57]	1.14 [0.63, 2.08]
p	0.001	0.001	<0.001	<0.001	0.688	0.198
Vegetarianism (ref: no)						
Yes	1.76 [1.27, 2.44]	1.82 [1.25, 2.66]	1.85 [1.34, 2.54]	1.85 [1.30, 2.64]	1.06 [0.73, 1.53]	1.39 [0.92, 2.09]
p	0.001	0.002	<0.001	0.001	0.769	0.116

Table shows odds ratio estimates from univariable and multivariable regression models predicting binary indicators of problematic substance use. For continuous exposure (i.e., dietary patterns), estimates indicate odds ratio for a 1 SD change. For categorical dietary measures, odds ratios are given relative to the stated baseline reference level.

The individual foods comprising the dietary patterns were then evaluated. Less frequent meat consumption and greater pulse and meat substitute consumption were each associated with increased risks of adolescent substance use, but no other foods showed consistent relationships (Table 3, Fig. 1, Tables S3 and S4), with the exception of increased rice, pasta, and nuts consumption being associated with cannabis use measures. Dietary intakes at 13 years of age showed similar specificities for individual foods, but less frequent meat consumption did not have a dose–response pattern to substance use risk at 15 years of age (Fig. S1).

Parent–Child Relationships, Substance Abuse, and Maternal Diets

Adverse parent–child relationships (less monitoring, greater conflict, less child disclosure) were strongly associated with increased risks of adolescent substance use (Table S5). We posited that the maternal "vegetarian" pattern (or low meat consumption) might be associated with a particular style of parenting that might explain the increased risks of adolescent substance use. However, parent–child relationships were not related to either the "vegetarian" dietary pattern or meat consumption (Table S6), and henceforth, we rejected this explanation.

Iron

Biomarkers of iron status included: the first hemoglobin measure taken in pregnancy, cord iron, cord blood ferritin, and child hemoglobin at 7-, 9-, and 11-year clinics. Maternal prenatal iron supplementation was considered alone and including level of meat consumption. Interactions between maternal meat consumption and a functional single nucleotide polymorphism (SNP) variant in the hemochromatosis (*HFE*) gene (rs1800562) and 8 SNP variants in the transferrin

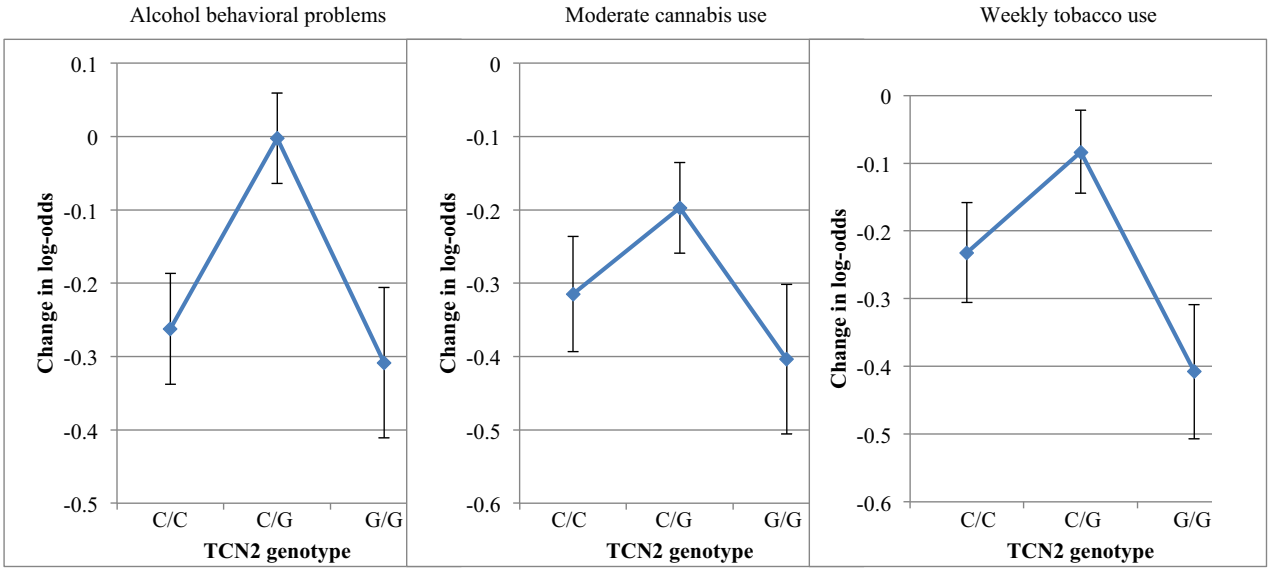

Fig. 2. Effect of higher maternal meat consumption in pregnancy on offspring substance use outcomes differentiated by maternal genotype for *TCN2* rs1801198. The effect of greater meat consumption by mothers (5 categories) and lower risk of substance abuse among their adolescents (lower log odds ratios) are stratified by maternal genotype for *TCN2 (*rs1801198). Diamonds and error bars (change in log-odds, 95% CI) indicate negative association between adverse substance use outcomes at 15 years of age. The cytosine (C) nucleotide encodes a proline (Pro) residue at amino acid 259 in the mature protein. The guanine (G) nucleotide encodes an arginine (Arg). The transcobalamin transporter is dimeric with suboptimal structural matching and transport function among heterozygous pairs, less responsive to maternal cobalamin intake. Group sizes were as follows: C/C: 30.7%, C/G: 50.1%, G/G: 19.2%.

receptor (*TFRC*) gene (rs9820939, rs570, rs714602, rs3817672, rs4927868, rs6583288) were evaluated. In summary, neither maternal nor child measures of iron status, nor maternal iron supplementation nor variants in iron metabolism, were robustly associated with adolescent substance abuse risk patterns.

TCN2

Offspring whose mothers carried homozygous alleles (CC or GG) of a sequence variant in *TCN2* (rs1801198) identified by Bonilla and colleagues (2012) showed a lower likelihood of adverse substance use that varied directly with higher meat consumption (Fig. 2 and Table S7). Among offspring of heterozygous *CG* mothers, in contrast, there was no pattern of meat consumption having any substantial modification of substance use The patterns of diet-modified response among homozygotes compared to the absence of a pattern of response among heterozygotes were common across all 10 measures of adverse substance abuse (Table S7, Fig. S2). Risks attributable to cobalamin deficits during pregnancy for adverse adolescent alcohol, cannabis, and tobacco use were 14, 37, and 23%, respectively (Table S8).

DISCUSSION

Adverse behavioral consequences of heavy alcohol use (9.5%), moderate cannabis use (9.6%), and weekly tobacco smoking (10.3%) are common among British 15-year-olds. We found that higher scores on the maternal "vegetarian" dietary pattern during pregnancy were associated with greater likelihoods of substance misuse among their 15-year-old offspring. Among foods loading onto the "vegetarian" diet pattern, less frequent consumption of red meat, poultry, and meat products (added together) was associated with greater risks of adverse alcohol, cannabis, and cigarette use OR = 1.75, 95% CI = [1.23, 2.56], $p < 0.001$, OR = 2.70, 95% CI = [1.89, 4.00], $p < 0.001$, and OR = 1.85, 95% CI = [1.28, 2.63], $p < 0.001$, respectively, comparing consumption of "0 to 4 meat portions" to "15+ times per fortnight." Consistent dose–response patterns comparing lower maternal meat consumption to increased risk were found for all measures of adverse alcohol, cannabis, and tobacco use at 15 years of age (see Table S3). However, many of these dose–response relationships were nonsignificant. Greater consumption of pulses and soy protein foods (substitutes for meat proteins) was also associated with a pattern of greater risk of all adverse substance use outcomes. Maternal prenatal consumption of all other foods including fish, fruits, and vegetables was unrelated to adolescent substance use risk.

Socioeconomic differences (Bonilla et al., 2012; Northstone et al., 2008) associated with less meat consumption were unlikely to explain our findings. Diet–substance use results persisted after adding terms for socioeconomic factors and inclusion of these terms strengthened diet–substance use associations and added precision to the point estimates. We had also posited that less meat consumption might be associated with more permissive parenting styles and that less parental monitoring would increase risk of substance use. In contrast, we found that less maternal meat consumption was associated with greater parental monitoring and thus would be likely to be protective. In order to more definitively rule

out residual confounding from socioeconomic and other differences, we employed stratification by genetic variants. We evaluated genetic variants in transport proteins that are necessary for the biological activity for 2 nutrients, cobalamin and iron, that are both essential for optimal neurodevelopment and frequently insufficient among infrequent meat consumers and pregnant vegetarians (Fayet et al., 2014).

In order to evaluate causality and address problems of residual confounding, we evaluated the effects of genetic variants in genes encoding cobalamin transport proteins. Randomized controlled trials provide a close analogy; a single biological factor is isolated between groups by random assignment so as to be free from confounding differences in order to evaluate a potential causal effect. The biological factors we employ here are the genetic variants encoding transport proteins for specific nutrients disproportionally rich in meat (iron and cobalamin) (Bonilla et al., 2012) and necessary for their biological activity. For example, the impact of maternal variants coding for suboptimal transportation of cobalamin to tissue on their offspring can be compared to the impact of maternal variants coding for optimal transportation of cobalamin on their offspring. These genetic variants in transportation determine different levels of the biological activity of these nutrients in target tissues. These variants are randomly distributed throughout this population (see Table S9). Thus, they are closely analogous to drug and placebo intervention trials that achieve differing tissue levels or agents, excepting that here "adherence" (differing levels of biological activity) is determined by genotype expression and group assignments are perfectly masked. We note of course that additive effects of *MTHFR* on other genotypes also influence cobalamin levels. Because these genetic variants are naturally randomized across the social, economic, and other differences among this population (see Table S9), these and residual confounding influences are highly unlikely to affect group comparisons based on these functional variants. Thus, a causal role can be evaluated on the basis of randomization, blinding, and adherence virtually free from socioeconomic, cultural, and other influences (Davey Smith and Hemani, 2014).

Functional Variants in TCN2 *Encoding the Cobalamin Transport Protein, Transcobalamin 2*

We posited that if cobalamin insufficiency had a causal role, the effects of low meat consumption would differ when comparing mothers within strata of DNA sequence variants associated with delivery of cobalamin to the nervous system. For this purpose, we evaluated functional common SNPs within ALSPAC mothers that existed in the gene encoding cobalamin transport protein transcobalamin 2 (*TCN2*)—these DNA variants were identified by Bonilla and colleagues (2012). Up to 30% of cobalamin in circulation binds to TCN2 and this biologically active complex, referred to as holo-transcobalamin (holo-TC), is critical for delivery of cobalamin to all body tissues (Rothenberg and Quadros, 1995). A common *TCN2* polymorphism, rs1801198, yields transition from cytosine to guanine at nucleotide base 776 (TC 776 C>G) yielding proline-to-arginine replacement of residue 259. In cohorts of Northern and Western European ancestry (HAPMAP-CEU), prevalence of the *GG, CG*, and *CC* variants is estimated to be 33%, 43%, and 23%, respectively (dbSNP, accessed February 20, 2015) (Afman et al., 2001). Because TCN2 is a binding and transport protein, both polymorphisms and dietary intakes may influence circulating homocysteine, holo-TC levels, and cobalamin concentrations (von Castel-Dunwoody et al., 2005; Zinck et al., 2015).

Here, we found that the effects of higher meat consumption during pregnancy on adolescent outcomes were specifically mediated by a functional variant in the *TCN2* allele (776C>G, rs1801198). Higher meat consumption was associated with substantially lower risk of substance use among the offspring of homozygous *CC* and *GG* mothers, but not among heterozygous *CG* mothers. Several investigators have reported similar biochemical patterns comparing the heterozygous to homozygous genotypes; these include elevated homocysteine (Alessio et al., 2007; von Castel-Dunwoody et al., 2005; Castro et al., 2010), lower cobalamin (von Castel-Dunwoody et al., 2005), and lower holo-TC (von Castel-Dunwoody et al., 2005). Others have failed to find such results for homocysteine, cobalamin, or holo-TC (Riedel et al., 2011). One explanation for the differences comparing homozygous to heterozygous mothers could be that the transcobalamin receptor is dimeric (Fig. 3) and is optimally functional only with identical subunits (Bose et al., 1996). Namour and Guéant (2001) in response to McCaddon and colleagues (2001) suggested that the Pro/Arg heterodimer isoform resulting from 776C>G, rs1801198 manifests

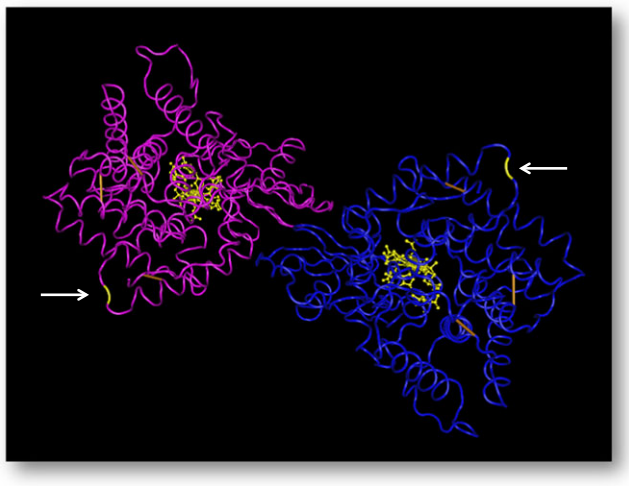

Fig. 3. TCN2 dimer (holo-transcobalamin) highlighting variant at amino acid position 776 (yellow). Wire frame structures of the transcobalamin *TCN2* dimer with cobalamin ligand represented in yellow at the center of each monomer. White arrows indicate amino acid residue 776, the site of proline-to-arginine transition consequent to DNA sequence variant rs1801198 (C>G). Disulfide bonds are represented in orange.

lower affinity for transcobalamin than either Pro/Pro or Arg/Arg homodimers and may explain differences in homocysteine levels. However, no biological substantiation of this suggestion was reported. Our findings indicate that insufficient tissue delivery of cobalamin may be both a plausible mechanism for impaired neurodevelopment consequent from less frequent maternal meat consumption and may be unaffected by residual confounding. However, we wish to emphasize that there is no consensus in the literature regarding the differences in biological activities of the transcobalamin products of *TCN2* homodimers compared to those of *TCN2* heterodimers and that this interpretation is therefore speculative.

Putative Mechanisms of Cobalamin Insufficiency in Substance Abuse Risk

Moderate cobalamin deficiencies in pregnancy and resulting elevations of homocysteine and hypermethylation (Gadgil et al., 2014) could potentially adversely impact neurodevelopmental processes relevant to substance abuse risk (Vaiserman, 2013) via homocysteine toxicity to dopaminergic neurons (Lipton et al., 1997) and global changes to DNA brain methylation (Sable et al., 2015). DNA methylation of quantitative trait loci appears to underlie some of the association of genetic variation with alcohol dependence (Zhang et al., 2014). Profound impairments in myelination were observed in the earliest autopsies for severe cobalamin deficiencies, and some evidence implicates impaired development of myelination in the frontal cortex as a risk factor for substance abuse (Acheson et al., 2014). Unfortunately, not enough is known about the impacts of moderate compared to severe deficits in cobalamin levels in human pregnancies on dopaminergic development, DNA methylation, and myelination deficits or the contribution of these processes to residual neurodevelopmental risks for substance abuse.

Strengths and Weaknesses

Although this is a well-characterized longitudinal cohort representative of the British population, differential attrition among families of low socioeconomic status may bias the findings. Here, we evaluate only early-onset substance use at 15 years of age and not longitudinal addictive phenotypes. However, the similar patterns of findings in multiple domains of substance abuse suggest impacts on common underlying neurobiological mechanisms in addiction. The effects of low meat consumption on increased substance use were not isolated to pregnancy, and lower meat consumption at 13 years of age was also associated with substance use outcomes at 16 years of age, but without a dose–response pattern and with lesser magnitude. FFQs are susceptible to differential misclassification expected to attenuate association measures. We have also not ruled out the potential contributions of adverse effects of pulses or soy meat substitutes. The use of Mendelian randomization techniques permitted an evaluation of gene-based causality unaffected by any social or residual confounding. Our probe set did not allow us to examine the rare variants in *TCN2*. We did not have measures of cobalamin, methyl-malonyl-CoA, or holo-TC among the mothers during pregnancy. Finally, we did not confirm, but cannot rule out, a contribution of low iron status. We did not create a model of all factors contributing to increased adolescence substance use risk.

CONCLUSIONS

This study identifies low meat consumption in the prenatal period as potentially modifiable risk factor for adolescent substance use. In identifying vitamin B12 insufficiencies as highly likely to have a contributing role to our findings, greater meat consumption need not be advised to modify this risk. For example, fortification of foods with vegetarian sources of cobalamin and more widespread use of supplements may be low cost and readily feasible interventions.

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AUTHORS' CONTRIBUTIONS

JRH, JH, JPSG, and JG designed the research. JRH and JH performed phenotypic analysis and JPSG genetic analysis. PME and KN provided essential dietary instruments and analyses. MS designed phenotypic characterizations. JRH, JPSG, JG, PME, KN, JMD, MS, and JH contributed to data interpretation and writing of the manuscript.

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CONFLICT OF INTEREST

The authors report no conflict of interest with regard to this research.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Missing data considerations.

Table S1. Principle component analysis results: maternal diets during pregnancy.

Table S2. Associations between socioeconomic characteristics and dietary pattern scores.

Table S3. Unadjusted results for maternal dietary intakes during pregnancy and seven additional adolescent substance use outcomes.

Table S4. Other maternal foods and adolescent substance use outcomes.

Table S5. Association between parental child interaction measures and substance use outcomes.

Table S6. Correlations between maternal dietary patterns in pregnancy and parent–child relationship.

Table S7. Effect of maternal meat consumption in pregnancy across strata of maternal genotype measures.

Table S8. Attributable risk.

Table S9. Sociodemographics comparing maternal *TCN2* (rs1801198) variants.

Fig. S1. Offspring diet at age 13 (maternal report) versus offspring substance use at age 15.

Fig. S2. Effect of higher maternal meat consumption in pregnancy on offspring substance use outcomes differentiated by maternal genotype for *TCN2* rs1801198.